Fragmentation of Deprotonated N-Benzoylpeptides: Formation of Deprotonated Oxazolones

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The fragmentation reactions of deprotonated N-benzoyl peptides, specifically hippurylglycine, hippurylglyclyclycine, and hippurylphenylalanine (hippuryl = N-benzoylGly) have been studied using MS^2 and MS^3 experiments as well as deuterium labeling. A major fragment ion is observed at *m/z* 160 ($[C_9H_6NO_2]^-$) which, upon collisional activation, mainly eliminates CO_2 indicating that the two oxygen atoms have become bonded to the same carbon. This observation is rationalized in terms of formation of deprotonated 2-phenyl-5-oxazolone. Various pathways to the deprotonated oxazolone have been elucidated through MS^3 experiments. Fragmentation of deprotonated N-acetylalanylalanine gives a relatively weak signal at *m/z* 112 which, upon collisional activation, fragments, in part, by loss of CO_2 leading to the conclusion that the *m/z* 112 ion is deprotonated 2,4-dimethyl-5-oxazolone. (J Am Soc Mass Spectrom 2004, 15, 446–456) © 2004 American Society for Mass Spectrometry

A common fragmentation reaction of collisionally activated protonated peptides involves cleavage of an amide bond [1–4]. When the charge resides on the C-terminal fragment a protonated amino acid (y_1'') or smaller protonated peptide (y_n'') ion is formed [5, 6]. When the charge resides on the N-terminal fragment the b ions formed, rather than having the expected acylium ion structure, have, in many cases, cyclized to form a protonated oxazolone [7–12]. Recent work by O'Hair and co-workers [13] suggests that in some cases alternative cyclic structures may be more stable than the oxazolone structure and thus, may be preferentially formed. Formation of oxazolone structures also rationalizes the observation that, while b_1 ions rarely are formed by cleavage of the first amide bond, N-acylation of the peptide frequently leads to cleavage of the N-terminal peptide amide bond since stable oxazolones can be formed [7, 9, 14].

Nominal amide bond cleavage also occurs for deprotonated peptides, as illustrated in Scheme **1**, where the y_n ions are deprotonated amino acids (y_1) or peptides (y_n) and the $''b_n$ ions bear two fewer hydrogens than the corresponding b_n ions formed from protonated peptides. Formation of $''b_n$ ions was first noted by Heerma and co-workers [15, 16] and elaborated upon by Bowie and co-workers [17–19]. The latter authors proposed the mechanism outlined in Scheme **2** for formation of $''b_2$ ions, designating the pathway leading to the deprotonated amino acid as α-cleavage and that leading to the charged N-terminal fragment as β-cleavage. From a low-energy CID study of deprotonated tripeptides, Harrison [20] proposed a similar direct cleavage of the amide bond but suggested that the charged N-terminal fragment had cyclized to form a deprotonated oxazolone. More recent studies [21] have shown that a major pathway to $''b_2$ ions from deprotonated tripeptides involves loss of a neutral amine from the a_3 ($[M - H - CO_2]^-$) ion, as illustrated in Scheme **3**, where again it is proposed that the $''b_2$ ion is a deprotonated oxazolone. The initial proton transfer reaction in Scheme **3** (and in later schemes) is undoubtedly exothermic but most likely involves a rotational barrier similar to the rotational barriers observed [22] in the fragmentation of a_2 ions derived from deprotonated dipeptides. Recent ab initio calculations in this laboratory [23] show that the $''b_2$ ion structure proposed in Scheme **2** readily rearranges over low energy rotational barriers to form the deprotonated oxazolone of Scheme **3**, a cyclization which is ca. 23 kcal mol^{-1} exothermic. However, a N-deprotonated diketopiperazine is ca. 10 kcal mol^{-1} more stable than the deprotonated oxazolone. In the present work we report a study of the fragmentation reactions of deprotonated N-benzoylpeptides using MS^2 and MS^3 collision-induced dissociation (CID) studies as well as isotopic labeling. The results provide compelling evidence for formation of deprotonated oxazolones.

Experimental

Initial CID studies were carried out using an electrospray/quadrupole mass spectrometer (VG Platform, Micromass, Manchester, UK) with CID in the interface

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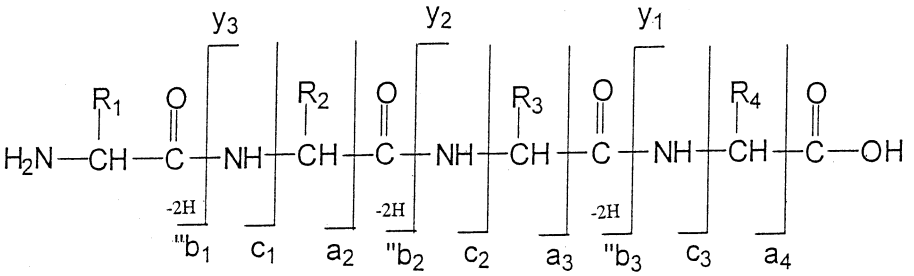
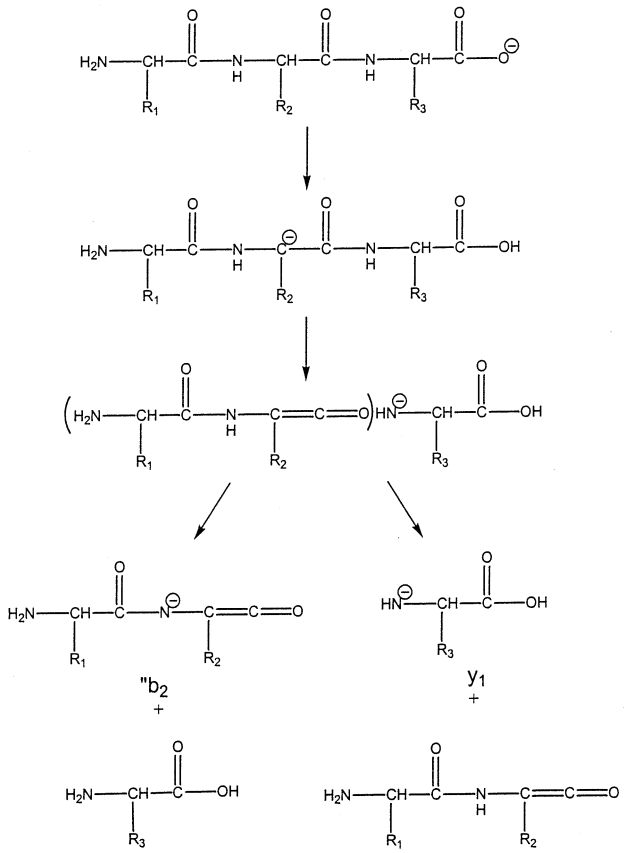
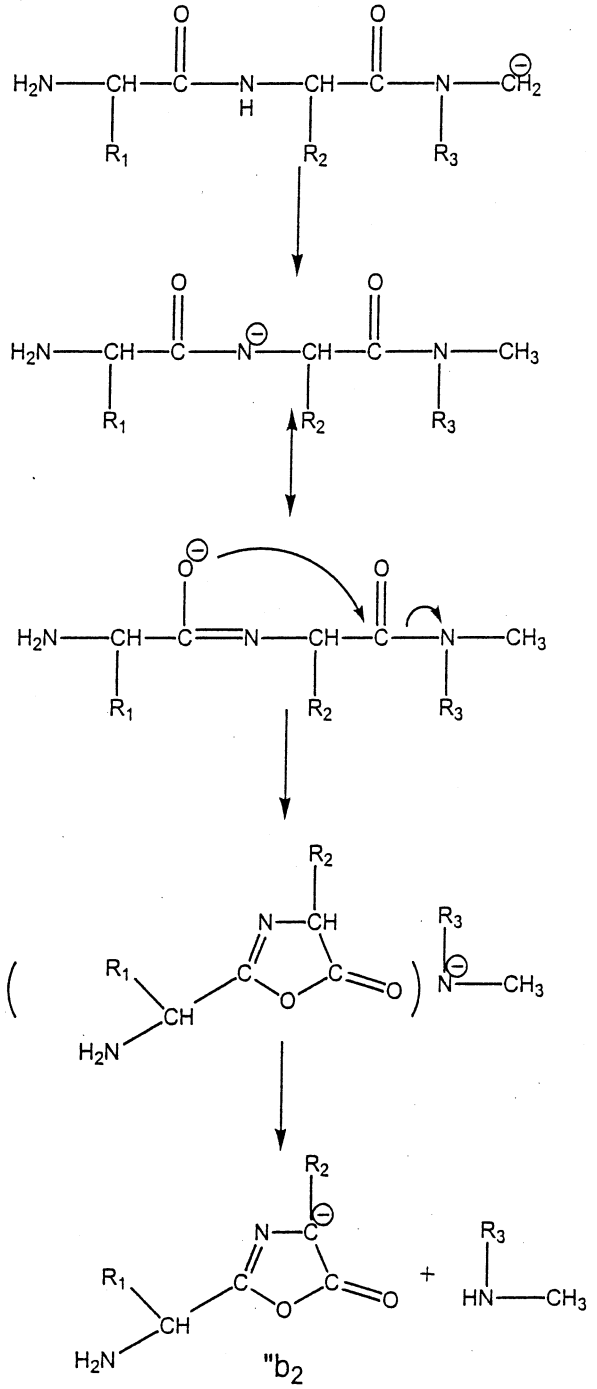


Scheme 1

region between the atmospheric pressure source and the quadrupole mass analyzer. It is well-known [24, 25] that CID can be achieved in this region, so-called cone-voltage CID, and it has been clearly established [26–28] that the average energy imparted to the decomposing ions increases as the field in the interface region increases. Recent studies [29–32] have shown that, by varying this field in steps, energy-resolved mass spectra [33] similar to those obtained by variable low-energy CID in quadrupole cells can be obtained. The results of these cone-voltage CID experiments are presented in the following as CID mass spectra at a set cone voltage. MS^2 and MS^3 experiments also were carried out using an electrospray/quadrupole/time-of-flight (QqTOF) mass spectrometer (QStar, MDS SCIEX, Concord, Can-



Scheme 2



Scheme 3

Table 1. CID of anions derived from hippurylglycine

fragment	*m/z* 235		*m/z*191		*m/z*	
m/z	20 eV	25 eV	20 eV	25 eV	20 eV	25 eV
217	1.3	2.0				
191	100	47.7				
173	5.7	6.3	9.5	5.7		
171		1.7	1.7	4.9		
160	91.6	100	100	98.0		
158		2.7	2.9	5.6		
147			4.2	1.5		
134	19.0	32.2	50.3	48.1		
132	1.5	4.8	5.6	15.3	5.6	3.5
121	2.7	4.1	7.6	5.5		
120	2.1	4.8	2.3	8.1		
116	4.1	25.3	23.1	91.7	100	100
113	26.6	52.6	73.0	100		
103			37.7	55.3		
85	2.7	7.6	10.7	20.3		
77	0.9	3.4	4.9	18.6	0.8	3.5
74	35.0	45.6				

ada). In the MS^3 experiments, CID in the interface region produced the fragment ion of interest which was mass-selected by the first quadrupole mass analyzer (Q) and underwent collisional activation in the quadrupole collision cell (q) with the ionic fragmentation products being analyzed by the time-of-flight analyzer.

With the single quadrupole instrument, ionization was by electrospray with the sample, at micromolar concentration in 1:1 CH_3CN/1% aqueous NH_3, being

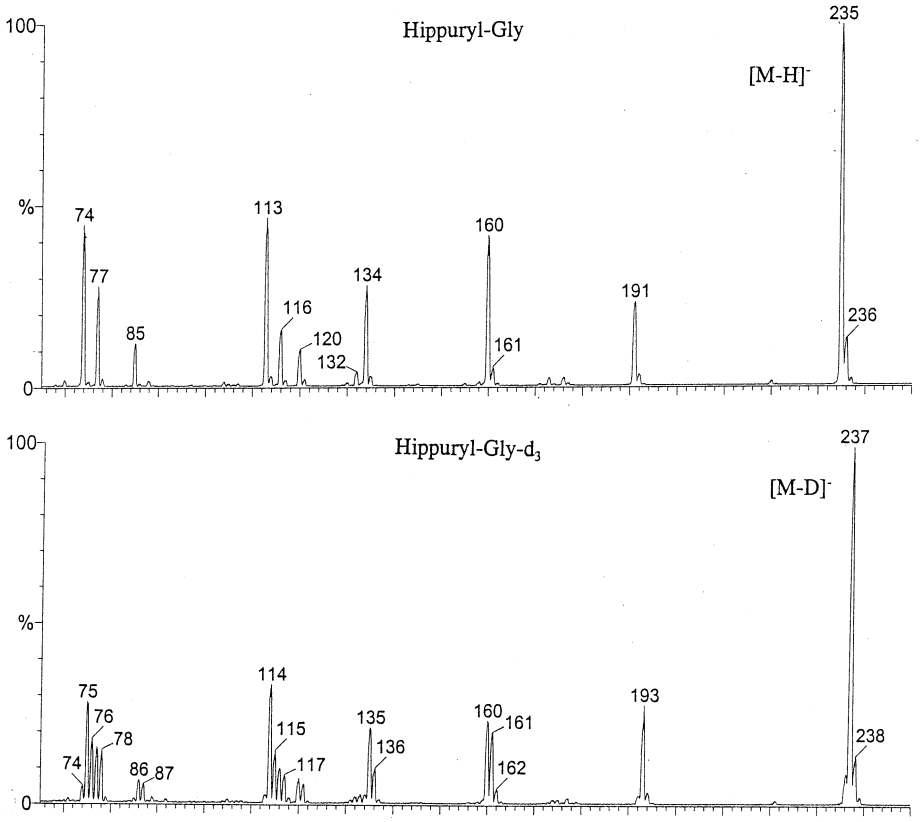
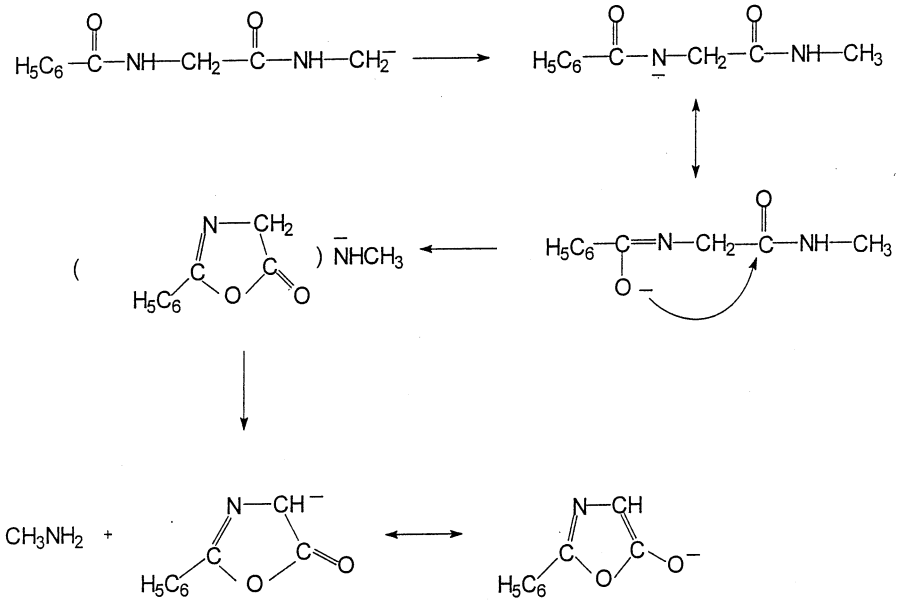


Figure 1. Comparison of the CID mass spectrum of deprotonated hippurylglycine with that of the $[M - D]^-$ ion of hippurylglycine-d_3. Cone voltage 50 V.



Scheme 4

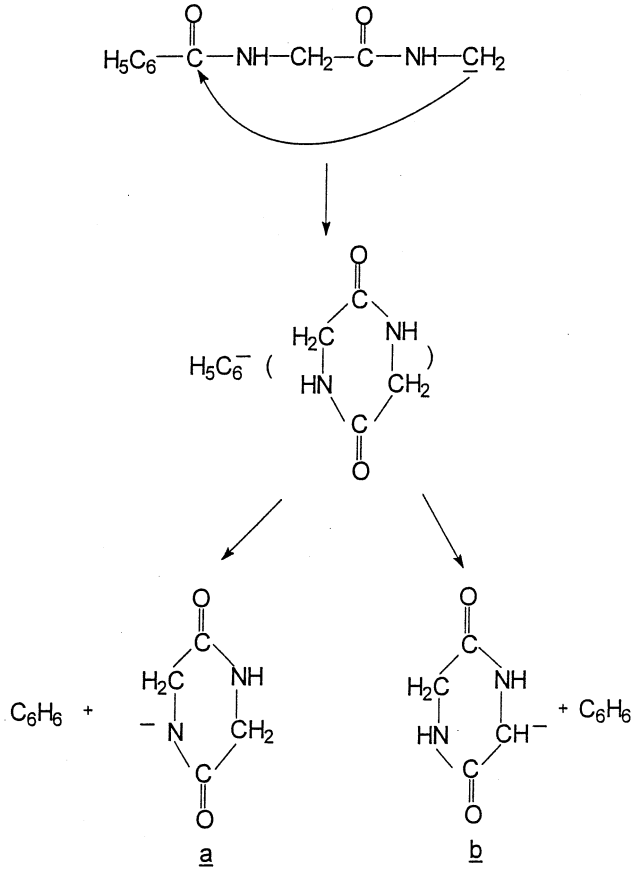
introduced into the source at a flow rate of 30 μL min^{-1}. The electrospray needle was held at −2.5 to 3.0 kV. Nitrogen, produced by a Whatman model 75-72 N_2 generator (Whatman Inc, Haverhill, MA) was used as both nebulizing gas and drying gas. By using 1:1 CD_3CN/1% ND_3 in D_2O as the electrospray solvent, the labile hydrogens were exchanged for deuterium and the $[M - D]^-$ ion was formed in the ionization process. Under these conditions no evidence was seen for back-exchange in the interface region although such back-exchange was observed when dry air was used as nebulizing and drying gas. Ionization was also by electrospray with the QqTOF instrument with the sample, in 1:1 CH_3OH/1% aqueous NH_3, being introduced into the source at a flow rate of 80 μL min^{-1}. Dry air was used as nebulizing and drying gas with N_2 being used as the collision gas under multiple collision conditions.

All peptide samples were obtained from BACHEM Biosciences (King of Prussia, PA). CD_3CN (99.8 atom% D) and D_2O (99.9 atom% D) were obtained from Cambridge Isotope Laboratories (Andover, MA) while ND_4OD (26% in D_2O, >99 atom% D) was obtained from CDN Isotopes (Pointe Claire, Quebec, Canada).

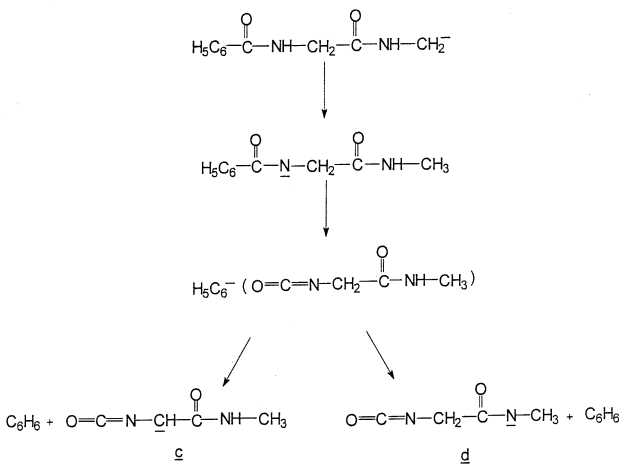
Results and Discussion

Table 1 records the CID mass spectra at two collision energies of deprotonated hippurylglycine (N-benzoylglycylglycine) as well as the CID mass spectra of the fragment ions of *m/z* 191 ($[M - H - CO_2]^-$) and *m/z* 160 as obtained on the QqTOF instrument. Figure 1 compares the CID mass spectrum obtained by cone-voltage CID of the deprotonated species with that of the $[M - D]^-$ ion of the peptide in which the labile hydrogens were exchanged for deuterium.

Of particular interest in the present context is the



Scheme 5



Scheme 6

fragment ion of *m/z* 160. The MS^3 experiments show that this fragment ion originates mainly by loss of 31 Da (CH_3NH_2) from the *m/z* 191 ($[M - H - CO_2]^-$) ion, a fragmentation reaction recently elucidated [21], although we cannot rule out the possibility of direct formation from the $[M - H]^-$ (*m/z* 235) ion by loss of neutral glycine as a minor pathway. The MS^3 experiments also show that the major fragmentation mode of the *m/z* 160 ion involves loss of CO_2 to give the fragment ion of *m/z* 116. Clearly, in the *m/z* 160 ion the two oxygens have become bonded to the same carbon. This observation is most readily rationalized in terms of formation of a deprotonated oxazolone (2-phenyl-5-oxazolone) by elimination of methylamine from the $[M - H - CO_2]^-$ as illustrated in Scheme 4. Elimination of CO_2 is at first surprising since it involves the breaking of a C–C bond which has at least partial double bond character; the driving force for the elimination of CO_2 is undoubtedly the high thermochemical stability of CO_2 although the structure of the *m/z* 116 ion is unclear. The pathway to the deprotonated oxazolone outlined in Scheme 4 predicts that no labile hydrogens should be retained by the fragment; however, as shown in Figure 1, there is appreciable retention of one labile hydrogen in the fragment. At the same time we note that there is appreciable incorporation of one labile hydrogen in the phenyl anion (*m/z* 77) indicating that there is interchange of labile hydrogens and a phenyl hydrogen although the mechanism and timing are not clear. That the deuterium incorporation in the phenyl ring does not occur in the liquid phase follows from the clean shift of the deprotonated species to *m/z* 137 (Figure 1) in the deuterated solvent.

A second major fragment ion arising from the *m/z* 191 ion is seen at *m/z* 113, corresponding to elimination of neutral benzene from the $[M - H - CO_2]^-$ ion. A plausible mechanism for benzene elimination is shown in Scheme 5 involving formation of an intermediate complex of a phenyl anion and a neutral diketopiperazine (cyclo-GlyGly). Within this complex, proton abstraction may occur either from the nitrogen or the carbon to form the isomeric deprotonated diketopiperazines **a** and **b**. Our ab initio calculations [23] indicate that the C-deprotonated diketopiperazine **b** is ca. 10 kcal mol^{-1} higher in energy than the N-deprotonated species **a**, but that both the N–H and C–H bonds in the

Table 2. CID of anions derived from hippurylglycylglycine

fragment	*m/z* 292		*m/z* 248		*m/z* 191	
m/z	20 eV	25 eV	20 eV	25 eV	20 eV	25 eV
274	4.2					
248	97.6	29.8				
230	11.6	5.7	18.1	8.1		
217	42.3	8.5				
191	100	100	100	100		
178	8.5	3.6				
177	5.6	6.2		2.2		
173	7.1	6.3			8.7	9.8
160	53.6	51.0	45.8	41.0	100	88.4
158		2.8		1.6	2.8	6.3
134	8.5	1.4	6.8	10.0	49.9	55.4
132					5.7	15.5
131	19.5	13.4				
121					7.6	7.0
120	54.7	59.9	16.8	28.0	66.4	64.3
116		2.1			25.7	84.7
113	22.6	28.0	20.2	22.4	70.8	100
103					16.1	14.1
87	6.3	11.2	4.1	8.4		
85					10.4	26.1
77					7.6	18.3
74	22.8	20.3				
70					5.2	7.3

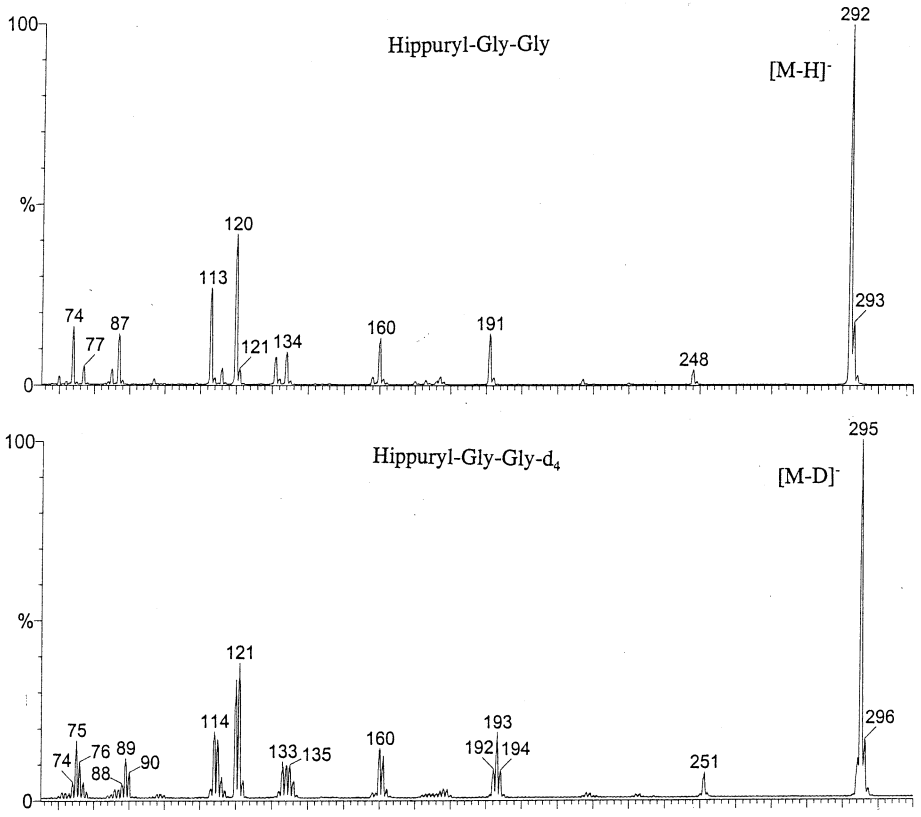
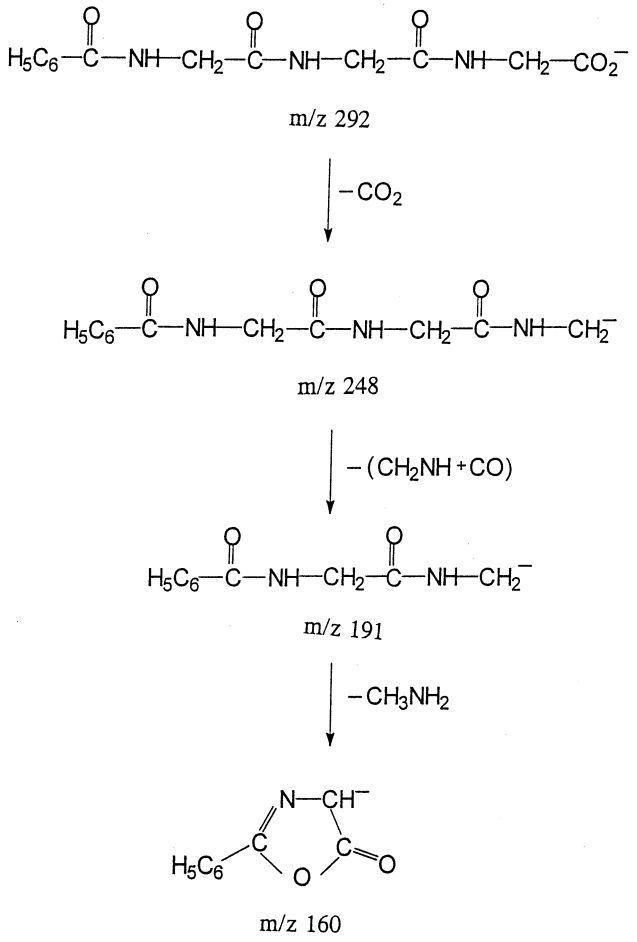


Figure 2. Comparison of the CID mass spectrum of deprotonated hippurylglycylglycine with that of the $[M - D]^-$ ion of hippurylglycylglycine-d_4. Cone voltage 50 V.

diketopiperazine are considerably more acidic than the C–H bond in benzene making proton abstraction from either position exothermic. An alternative pathway to *m/z* 113, outlined in Scheme **6**, involves proton abstraction from the N-terminal amide position leading to the phenyl anion/neutral complex shown. Ab initio calculations [23] show that the isocyanato neutral in the complex is only 6–7 kcal mol^{-1} higher in energy than the diketopiperazine of Scheme **5** and that the anion **d** derived by N–H deprotonation has essentially the same energy as the N-deprotonated diketopiperazine **a**. The anion **c** is 17.5 kcal mol^{-1} higher in energy than **a** or **d** but proton abstraction from this position by the phenyl anion remains exothermic [23]. From the spectra of Figure 1 it is clear that the *m/z* 113 ion incorporates one and, to some extent, two labile hydrogens. Given the apparent interchange of a phenyl hydrogen with a labile hydrogen this observation provides no significant information. Unfortunately, we were unable to carry out MS^3 experiments on the *m/z* 113 fragment ion because of a strong and continual background signal at *m/z* 113 ($[CF_3COO]^-$) in the QqTOF instrument. At the present time we are not able to clearly elucidate the pathway(s) to *m/z* 113 and further studies of related compounds are under way.

Two further fragment ions of note in the fragmentation of deprotonated hippurylglycine are the y_1 ion, deprotonated glycine, at *m/z* 74 and the ion signal at *m/z* 134 ($[C_6H_5C(=O)NHCH_2]^-$) which presumably arises by loss of CH_2=NH + CO from the $[M - H - CO_2]^-$ (*m/z* 191) ion.

The *m/z* 160 and *m/z* 113 ions are prominent in the CID mass spectrum of deprotonated hippurylglycylglycine (N-benzoyl-triglycine). MS^2 and MS^3 CID mass spectra are presented in Table 2 while, in Figure 2, the CID mass spectrum of the $[M - H]^-$ ion is compared with that of the $[M - D]^-$ ion of the peptide in which the labile hydrogens have been exchanged for deuterium. The *m/z* 160 ion gave a CID mass spectrum (not shown) very similar to that of the *m/z* 160 ion derived from deprotonated hippurylglycine (Table 1) with the main fragmentation mode being loss of CO_2 consistent with a deprotonated 2-phenyl-5-oxazolone structure. The CID mass spectra of Table 2 show that a major route to the *m/z* 160 ion involves the reaction sequence illustrated in Scheme **7** although some formation of *m/z* 160 directly from $[M - H]^-$ by elimination of neutral diglycine cannot be discounted. It is also possible that the *m/z* 160 product may arise directly from the $[M - H - CO_2]^-$ by elimination of neutral glycine methylamide. Other ions of interest in the fragmentation of $[M - H]^-$ are the y_1 ion at *m/z* 74 and the y_2 ion at *m/z* 131. The *m/z* 191 ion is predicted in Scheme **7** to have the same structure as the *m/z* 191 ($[M - H - CO_2]^-$) ion



Scheme 7

derived from hippurylglycine and, indeed, the two ion show very similar CID mass spectra with the exception that the former ion shows (Table 2) a much more pronounced signal at *m/z* 120 ($[C_6H_5CONH]^-$); the reasons for this difference are not obvious.

Deprotonated hippurylphenylalanine (N-benzoylglycylphenylalanine) also shows a fragment ion of *m/z* 160 (Table 3 and Figure 3). This ion shows (Table 3) the characteristic loss of CO_2 indicating the deprotonated oxazolone structure. However, CID of the $[M - H - CO_2]^-$ (*m/z* 281) ion does not lead to the deprotonated oxazolone by the pathway analogous to Scheme **4** involving elimination of 2-phenylethyl amine from the $[M - H]^-$ ion. Rather the $[M - H - CO_2]^-$ ion (*m/z* 281) fragments essentially completely by elimination of 104 Da (C_8H_8, styrene?) to give *m/z* 177 which also is the base peak in the CID mass spectrum of $[M - H]^-$. Presumably, elimination of C_8H_8 from the $[M - H - CO_2]^-$ ion is a lower energy fragmentation route than elimination of 2-phenylethyl amine. A fragment ion of *m/z* 177 also may arise by direct loss of cinnamic acid from the $[M - H]^-$ ion, a fragmentation reaction previously elucidated [34] for deprotonated peptides containing C-terminal phenylalanine. The *m/z* 177 ion fragments, in part, by

Table 3. CID of anions derived from hippurylphenylalanine (20 eV collision energy)

fragment *m/z*	*m/z* 325	*m/z* 281	*m/z* 177	*m/z* 160
307	0.8			
281	26.9			
264	2.1			
203	1.1			
189	1.4			
177	100	100		
164	52.5			
160	28.6	1.7	100	
159	5.2	3.4	77.3	
147	7.2			
134	1.4	0.8	42.7	
132			4.9	4.3
120	1.6			
116			32.6	100
99			49.4	
77			7.7	1.3
71			7.4	

elimination of NH_3 to give deprotonated 2-phenyl-5-oxazolone (*m/z* 160); the labeling results (Figure 3) show that in this case the deprotonated oxazolone contains no labile hydrogens. The apparent pathway to *m/z* 160 is outlined in Scheme **8**. The *m/z* 177 ion also fragments, in part, by elimination of H_2O, a reaction previously observed [34] for ions formed by elimination of cinnamic acid from deprotonated peptides containing C-terminal phenylalanine. Deprotonated phenylalanine (y_1 ion) also is observed at *m/z* 164.

The formation of deprotonated oxazolones is not limited to N-benzoyl derivatives. Fragmentation of the $[M - H]^-$ ion derived from N-acetyl-Ala-Ala-OH shows (Table 4 and Figure 4) y_1 (*m/z* 88) and *m/z* 115 ($[M - H - CH_2CO - CO_2]^-$) as the major fragment ions but of particular interest is the relatively weak signal at *m/z* 112 which appears to arise by elimination of neutral alanine from the $[M - H]^-$ ion. CID of the *m/z* 112 ion showed (Table 4) loss of CH_3 (*m/z* 97) and loss of CO (*m/z* 84) but also a significant signal at *m/z* 68 corresponding to elimination of CO_2 which we take to be characteristic of a deprotonated oxazolone structure. Whereas elimination of CO forms the base peak in the fragmentation of *m/z* 112 it is only a minor peak in the fragmentation of the *m/z* 160 ions derived from the benzoyl derivatives. It appears that the phenyl group stabilizes the fragment ion resulting from CO_2 loss to a much greater extent than does the methyl group. Exchange of the labile hydrogens for deuterium (Figure 4) shows that the major part of the ion signal does not incorporate labile hydrogens as expected. Note again that CID of the $[M - H - CO_2]^-$ ion (*m/z* 157) did not result in formation of *m/z* 112 by a pathway analogous to Scheme **3**; it is not clear why this is so. The MS^3 experiments show that the *m/z* 115 ion arises by two pathways, initial loss of ketene

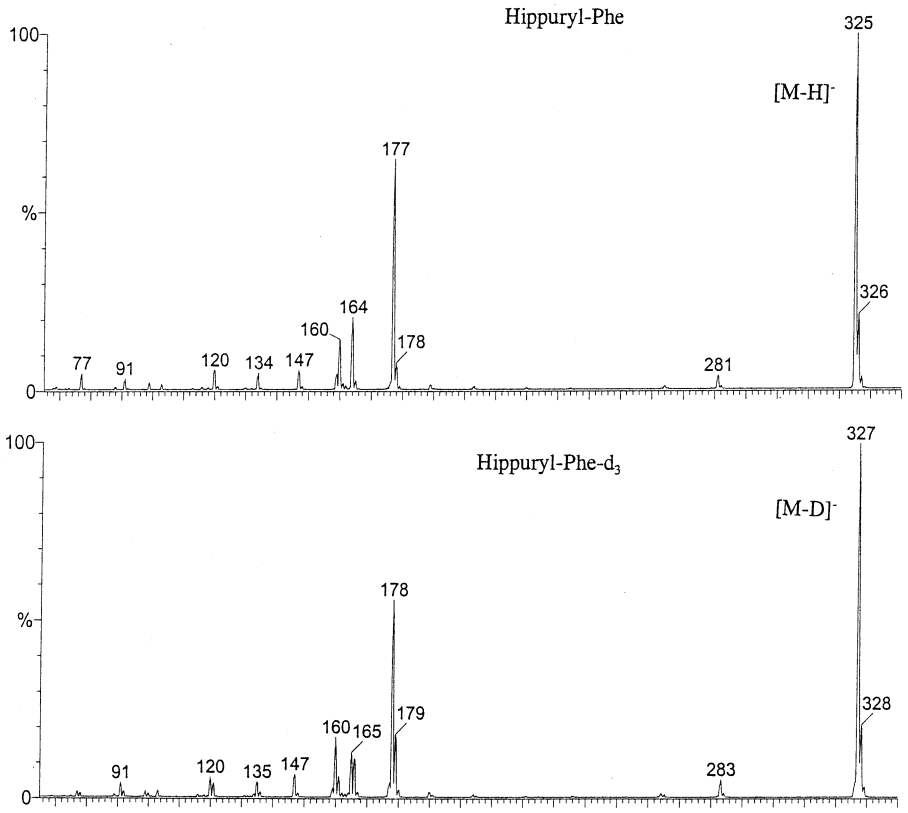
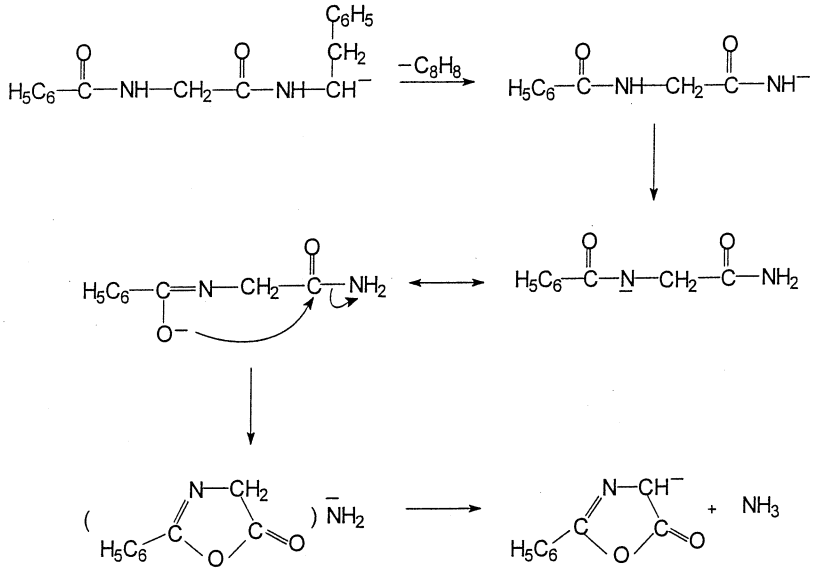


Figure 3. Comparison of the CID mass spectrum of deprotonated hippurylphenylalanine with that of the $[M - D]^-$ ion of hippurylphenylalanine-d_3. Cone voltage 50 V.

followed by loss of CO_2 and initial loss of CO_2 followed by loss of ketene. The major part of the *m/z* 115 signal incorporates two labile hydrogens as expected.

Conclusions

The present work has shown that deprotonated N-benzoyl (i.e., hippuryl) peptides fragment, in part, to



Scheme 8

Table 4. CID of anions derived from deprotonated N-acetylalanylalanine (20 eV collision energy)

fragment *m/z*	*m/z* 201	*m/z* 159	*m/z* 157	*m/z* 112
159	17.4			
157	5.6			
141		2.0		
129	2.1			
117		3.2		
115	69.3	100	100	
112	12.5			
99		3.6		
98		21.2		
97				10.7
89			16.3	
88	100	63.5		
87		12.3		
84				100
72		8.2		
70		3.7		
68				26.6
58				10.7

form an ion of *m/z* 160 which has been identified as deprotonated 2-phenyl-5-oxazolone on the basis of the dominant loss of CO_2 upon collisional activation. The pathways to this product are varied. For deprotonated hippurylglycine the *m/z* 160 product originates largely by elimination of CH_3NH_2 from the $[M - H - CO_2]^-$ (*m/z* 191) ion, a reaction pathway recently elucidated [21], although we cannot rule out some formation directly from the $[M - H]^-$ ion. For deprotonated hippurylglycylglycine a major pathway involves loss of 57 Da (probably CH_2=NH + CO) from $[M - H - CO_2]^-$ to produce an ion of *m/z* 191 which subsequently eliminates CH_3NH_2, as observed for deprotonated hippurylglycine. However, we cannot rule out the possibility that some of the oxazolone product originated directly from the $[M - H - CO_2]^-$ ion (involving nominal elimination of glycine methylamide) or, possibly, directly from the $[M - H]^-$ ion. For deprotonated hippurylphenylalanine the $[M - H - CO_2]^-$ ion preferentially eliminates a neutral of 104 Da (styrene?) to form an ion that is nominally deprotonated N-benzoylglycinamide; this species subsequently eliminates ammonia to form the deprotonated oxazolone. Again, direct formation from the $[M - H]^-$ ion remains a possible second pathway.

Formation of deprotonated oxazolones is not limited to benzoyl derivatives since deprotonated N-acetylalanylalanine, upon fragmentation, produces an ion of *m/z* 112 which we have identified as deprotonated 2,4-dimethyl-5-oxazolone from the characteristic loss of CO_2.

Finally, we note that protonated hippuryl peptides, such as hippurylglycine and hippurylphenylalanine,

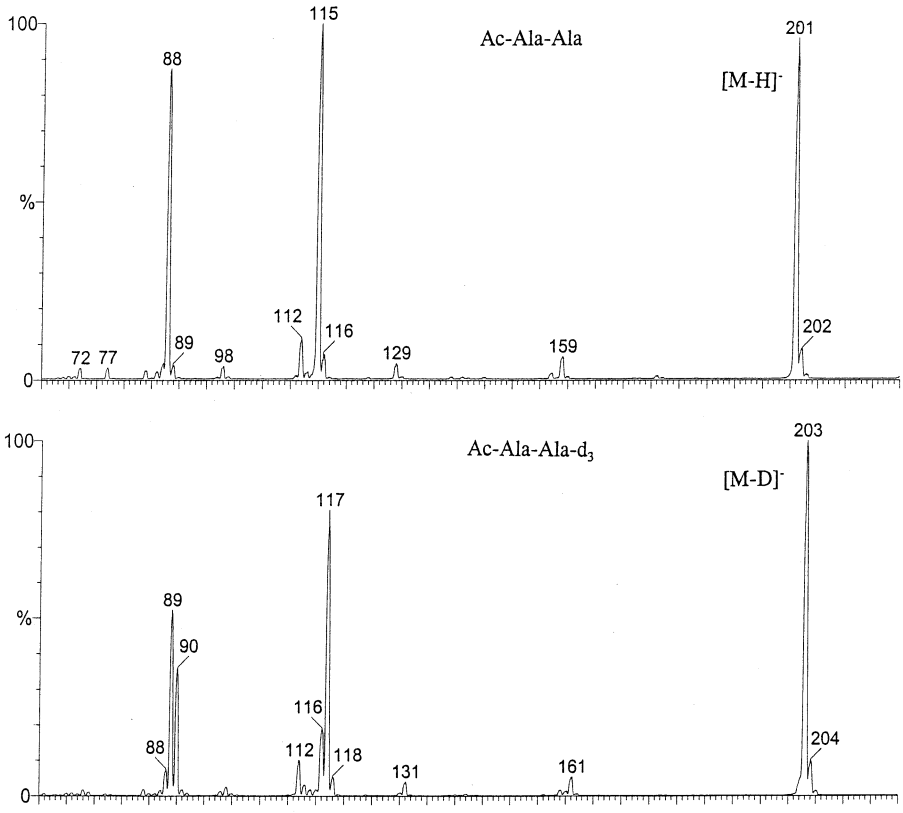


Figure 4. Comparison of the CID mass spectrum of deprotonated acetylalanylalanine with that of the $[M - D]^-$ ion of acetylalanylalanine-d_3. Cone voltage 50 V.

upon fragmentation form an ion of *m/z* 162 which has been identified as protonated 2-phenyl-5-oxazolone [7, 9]. Indeed, the tendency of peptides in the positive ion mode to form product ions containing five-membered rings has been discussed in detail [35]. It appears that there may be a similar tendency in the negative ion mode.

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References

1. Roepstorff, P.; Fohlman, J. Proposal for a Common Nomenclature for Sequence Ions in Mass Spectra of Peptides. *Biomed. Mass Spectrom.* **1984,** *11,* 601.
2. Biemann, K. Contribution of Mass Spectrometry to Peptide and Protein Structure. *Biomed. Env. Mass Spectrom.* **1988,** *16,* 99.
3. Biemann, K. Sequencing of Peptides by Tandem Mass Spectrometry and High-Energy Collision-Induced Dissociation. *Methods Enzymol.* **1990,** *193,* 455.
4. Papayannopoulos, I. A. The Interpretation of Collision-Induced Dissociation Tandem Mass Spectra of Peptides. *Mass Spectrom. Rev.* **1995,** *14,* 49.
5. Mueller, D. R.; Eckersley, M.; Richter, W. Hydrogen Transfer in Formation of "Y + 2" Sequence Ions from Protonated Peptides. *Org. Mass Spectrom.* **1988,** *23,* 217.
6. Cordero, M. M.; Houser, J. J.; Wesdemiotis, C. The Neutral Products Formed During Backbone Fragmentation of Protonated Peptides in Tandem Mass Spectrometry. *Anal. Chem.* **1993,** *65,* 1594.
7. Yalcin, T.; Khouw, C.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. Why are B Ions Stable Species in Peptide Mass Spectra? *J. Am. Soc. Mass Spectrom.* **1995,** *6,* 1165.
8. Yalcin, T.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. The Structure and Fragmentation of B_n ($n \geq 3$) in Peptide Mass Spectra. *J. Am. Soc. Mass Spectrom.* **1996,** *7,* 293.
9. Nold, M. J.; Wesdemiotis, C.; Yalcin, T.; Harrison, A. G. Amide Bond Dissociation in Protonated Peptides. Structures of the N-Terminal Ionic and Neutral Fragments. *Int. J. Mass Spectrom. Ion Processes* **1997,** *164,* 137.
10. Paizs, B.; Lendvay, G.; Vékey, K.; Suhai, S. Formation of b_2^+ Ions from Protonated Peptides: An ab Initio Study. *Rapid Commun. Mass Spectrom.* **1999,** *13,* 525.
11. Harrison, A. G.; Csizmadia, I. G.; Tang, T.-H. Structures and Fragmentation of b_2 Ions in Peptide Mass Spectra. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 427.
12. Rodriquez, C. F.; Shoeib, T.; Chu, I. K.; Siu, K. W. M.; Hopkinson, A. C. Comparison Between Protonation, Lithiation, and Argentination of 5-Oxazolones: A Study of a Key Intermediate in Gas-Phase Peptide Sequencing. *J. Phys. Chem. A* **2000,** *104,* 5335.
13. Farrugia, J. M.; O'Hair, R. A. J.; Reid, G. E. Do All b_2 Ions have Oxazolone Structures? Mass Spectrometry and ab Initio Studies on Protonated N-Acyl Amino Acid Methyl Ester Model Systems. *Int. J. Mass Spectrom.* **2001,** *210/211,* 71.
14. Vaisar, T.; Urban, J. Gas-Phase Fragmentation of Mono-N-Methylated Peptides. Analogy with Solution-Phase Acid-Catalyzed Hydrolysis. *J. Mass Spectrom.* **1998,** *33,* 505.
15. Kulik, W.; Heerma, W. Fast Atom Bombardment Tandem Mass Spectrometry for Amino Acid Determination in Tripeptides. *Biomed. Env. Mass Spectrom.* **1989,** *18,* 9.
16. van Setten, D.; Kulik, W.; Heerma, W. Isomeric Tripeptides: A Study on Structure–Spectrum Relationship. *Biomed. Env. Mass Spectrom.* **1990,** *19,* 475.
17. Eckersley, M.; Bowie, J. H.; Hayes, R. N. Collision-Induced Dissociation of Deprotonated Peptides: Dipeptides and Tripeptides with Hydrogen and Alkyl α-Groups. *Org. Mass Spectrom.* **1989,** *24,* 597.
18. Waugh, R. J.; Bowie, J. H. A Review of the Collision Induced Dissociations of Deprotonated Dipeptides and Tripeptides. An Aid to Structure Determination. *Rapid Commun. Mass Spectrom.* **1994,** *8,* 169.
19. Bowie, J. H.; Brinkworth, C. S.; Dua, S. Collision Induced Fragmentations of the $[M - H]^-$ Parent Anions of Underivatized Peptides. An Aid to Structure Determination and Some Unusual Negative Ion Cleavages. *Mass Spectrom. Rev.* **2002,** *21,* 87.
20. Harrison, A. G. Sequence-Specific Fragmentation of Deprotonated Peptides Containing H or Alkyl Side Chains. *J. Am. Soc. Mass Spectrom.* **2001,** *12,* 1.
21. Harrison, A. G.; Siu, K. W. M.; El Aribi, H. Amide Bond Cleavage in Deprotonated Tripeptides: A Newly Discovered Pathway to $''b_2$ Ions. *Rapid Commun. Mass Spectrom.* **2003,** *17,* 869.
22. Chass, G. A.; Marai, C. N. J.; Harrison, A. G.; Csizmadia, I. G. Fragmentation Reactions of a_2 Ions Derived from Deprotonated Dipeptides—A Synergy Between Experiment and Theory. *J. Phys. Chem. A* **2002,** *106,* 9695.
23. Chass, G. A., Marai, C. N. J., Csizmadia, I. G., Harrison, A. G. A Hartree-Fock, MP2 and DFT Computational Study of the Structures and Energies of $''b_2$ Ions Derived from Deprotonated Peptides. A Comparison of Method and Basis Set Used on Relative Product Stabilities. *J. Molec. Struct. (THEOCHEM)*, in press.
24. Loo, J. A.; Udseth, H. R.; Smith, R. D. Collisional Effects on the Charge Distribution of Ions From Large Molecules Formed by Electrospray-Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1988,** *2,* 207.
25. Bruins, A. P. ESI Source Design and Dynamic Range Considerations. *In Electrospray Mass Spectrometry: Fundamentals, Instrumentation, and Applications;* Cole, R. B., Ed.; Wiley: New York, 1997, p 107.
26. Voyksner, T. D.; Pack, T. Investigation of Collisional-Activation Decomposition Processes and Spectra in the Transport Region of an Electrospray Single-Quadrupole Mass Spectrometer. *Rapid Commun. Mass Spectrom.* **1991,** *5,* 263.
27. Collette, C.; DePauw, E. Calibration of the Internal Energy of Ions Produced by Electrospray. *Rapid Commun. Mass Spectrom.* **1998,** *12,* 165.
28. Collette, C.; Drahos, L.; DePauw, E.; Vékey, K. Comparison of the Internal Energy Distribution of Ions Produced by Different Electrospray Sources. *Rapid Commun. Mass Spectrom.* **1998,** *12,* 1673.
29. Harrison, A. G. Energy-Resolved Mass Spectrometry. A Comparison of Quadrupole Cell and Cone Voltage Collision-Induced Dissociation. *Rapid Commun. Mass Spectrom.* **1999,** *13,* 1663.
30. van Dongen, W. D.; van Wijk, J. I. T.; Green, B. M.; Heerma, W.; Haverkamp, J. Comparison Between Collision Induced Dissociation of Electrosprayed Protonated Peptides in the

Up-Front Region and in a Low-Energy Collision Cell. *Rapid Commun. Mass Spectrom* **1999,** *13,* 1712.
31. Harrison, A. G. Fragmentation Reactions of Alkylphenyl Ammonium Ions. *J. Mass Spectrom.* **1999,** *34,* 1253.
32. Makowiecki, J.; Tolonen, A.; Uusitalo, J.; Jalonen, J. Cone Voltage and Collision Cell Collision-Induced Dissociation of Triphenylethylenes of Pharmaceutical Interest. *Rapid Commun. Mass Spectrom.* **2001,** *15,* 1506.
33. McLuckey, S. A.; Cooks, R. G. Angle- and Energy-Resolved Fragmentation from Tandem Mass Spectrometry. *In Tandem Mass Spectrometry;* McLafferty, F. W., Ed.; Wiley: New York, 1983, p 203.
34. Harrison, A. G. Effect of Phenylalanine on the Fragmentation of Deprotonated Peptides. *J. Am. Soc. Mass Spectrom.* **2002,** *13,* 1242.
35. Schlosser, A.; Lehmann, W. D. Five-Membered Ring Formation in Unimolecular Reactions of Peptides: A Key Structural Element Controlling Low-Energy Collision-Induced Dissociation of Peptides. *J. Mass Spectrom.* **2000,** *35,* 1382.